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Caloric Compensation in Hypothalamic Obese Rats[1]

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SMUTZ, E. R., E. HIRSCH AND H. L. JACOBS. *Caloric compensation in hypothalamic obese rats.* PHYSIOL. BEHAV. 14(3) 305–309, 1975. – The present study explored the influence of pelleted diets on adjustment to caloric dilution in hypothalamic obese rats. Medial hypothalamic lesioned and normal rats were maintained on a high fat diet until the static stage of hyperphagia was reached. They were given three pelleted diets which consisted of undiluted Noyes pellets and Noyes pellets diluted 25 or 50 percent with kaolin. In contrast to previous reports that hypothalamic obese rats do not compensate for caloric dilution of their diet, the obese animals showed as large an increase in food intake on the dilute pelleted diets as did the control animals. These results were interpreted by viewing texture as a dimension of palatability which influences food intake in hypothalamic obese rats.

Caloric compensation Hypothalamic obesity Food intake Texture

THE sensory properties of food exert very little controlling influence on caloric intake or body weight level in the neurologically intact animal with food and water freely available [1, 11, 22]. However, after damage to the ventromedial region of the hypothalamus the taste and texture of food become major determinants of the level of food intake and the degree of obesity that develops [4, 5, 16, 18, 19, 29].

The prepotent role of taste, texture and smell in controlling food intake in hypothalamic hyperphagic animals is also underscored by the response of these animals to caloric dilution. An animal in the static stage of hypothalamic hyperphagia will actively defend this elevated body weight by appropriate changes in food intake when weight loss is produced by food deprivation [2,10] or when weight gain is produced by forced feeding or daily insulin injections [10]. This elevated body weight is not defended when hypothalamic obese animals are confronted with a diet that is adulterated with a nutritionally inert material like cellulose or kaolin [12, 14, 24, 29]. Instead of increasing food intake to maintain a constant level of daily caloric intake these animals reduce bulk intake and lose weight compared to normal animals which show a proportional adjustment in food intake up to approximately 50% dilution.

The failure of hypothalamic hyperphagic animals to maintain caloric intake on diluted diets has been attributed to finickiness or over-reactivity to the stimulus properties of the diet [12]. It is usually argued that the addition of cellulose or kaolin to the diet reduces the palatability of the adulterated food. This interpretation is consistent with studies that show caloric compensation in hypothalamic hyperphagic rats when diets are diluted with water [28,32] or mineral oil [4] if these diluents do not influence palatability or enhance it. In the case of greasy diets that are adulterated with non-nutritive material the available evidence indicates that they are highly preferred to pellets [4] or powdered chow [9] even though their caloric density is lower. These observations suggest that palatability is an important determinant of the hyperphagic rat's response to dietary dilution.

The present experiment explores the influence of texture as a dimension of palatability and examines the response of rats with lesions of the medial hypothalamus to diluted diets that are presented in pelleted form. This dimension of food was chosen for examination because previous reports have shown that if the same diet is presented as pellets or powder to hypothalamic hyperphagic rats food intake and assymptotic body weight will be substantially higher on the pelleted form of the diet [5, 16, 19, 29].

METHOD

Animals

Twelve male and 12 female, albino Holtzman rats (Madison, Wisconsin) were used in this experiment. Within

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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each sex 2 groups of 6 animals were formed that were matched on the basis of initial body weights. The average weights of these groups at the time of surgery were 362 g for the males and 279 g for the females.

Procedure

Rats were individually housed in wire mesh cages (Fenco Co.) in a room that was on a 12 – 12 light-dark cycle (lights on at 8 a.m.). Temperature averaged 24 ± 1°C. Food was available in 5 cm diameter cups with lids that had 4.45 cm diameter openings in the center. Spillage was negligable with the diets employed. Water was continuously available in 250 ml calibrated bottles.

After a 2 week accommodation period in the laboratory all animals were operated on and maintained on a high fat diet (HF) that consisted of Purina chow and 25% corn oil (Mazola) by weight (4.96 kcal/g). Body weight, food intake and water intake were recorded daily at 10:00 a.m. This procedure was to have been continued until the body weights of the lesioned animals reached a stable plateau. However, three of the lesioned males died during the period of weight gain. To avoid the possibility of losing additional animals testing on the diluted diets was initiated 100 days after surgery. At this point the lesioned males had become rather obese but were still gaining weight at the rate of 2.8 g/day compared to 0.8 g/day for the control males. The lesioned females, on the other hand, had attained asymptotic levels of body weight approximately 3 weeks prior to the start of testing. For this reason the intake data of the males and females were analyzed separately.

The test diets consisted of 45 mg Noyes pellets (Lab Food A, P. J. Noyes Co., Lancaster, New Hampshire) diluted by the addition of kaolin. Three levels of pellet dilution were used: 0% kaolin (P–O), 25% kaolin (P–25), and 50% kaolin (P–50). The caloric densities of these diets are 3.65 kcal/g, 2.71 kcal/g and 1.83 kcal/g. These values were computed from the nutrient composition of Lab Food A using 9, 4 and 4 kcal/g for fat, protein and carbohydrate, respectively. Each diet was presented for two, 4 day periods in the following ascending-descending sequence: P–O, P–25, P–50, P–50, P–25, P–O.

Surgery

All groups were deprived of food approximately 18 hrs prior to surgery. Lesions were made in the experimental animals under sodium pentobarbitol anesthesia (35 mg/kg) by passing 2 mA of anodal current for 20 sec through a 0.25 mm dia. stainless steel electrode which was completely insulated except for 0.5 mm at the tip. Atropine (0.044 mg/kg) was also given to reduce bronchial secretions. A Kopf stereotaxic instrument was used to position the electrode, with reference to a flat skull, 2.4 mm posterior to bregma, 0.6 mm lateral to the center of the saggital sinus and 9.5 mm below the surface of the skull. Control animals were exposed to the same procedure up to the point of lowering the electrode into the brain. Following surgery the animals were injected with the stimulant pentylenetetrozol (50 mg/kg) and placed under a 100 W lamp until they recovered from the anesthesia. After testing was completed the animals were sacrificed with sodium pentobarbital and perfused with 0.9% saline. The brains were fixed with 10% Formalin. Sections were taken at 40 μ and every fifth section was stained with cresyl violet while every sixth section was stained with hematoxylin.

RESULTS

The lesions led to over-eating and excessive weight gain in all of the experimental animals. Figure 1 shows that the body weights of the lesioned females had reached a stable asymptote approximately 85 days after surgery whereas the lesioned males were still gaining weight at a faster rate than the control males 100 days after surgery when testing began.

The upper panel of Fig. 2 shows the average food intake data from the last 8 days of maintenance on HF as well as the intake data on each of the test diets. This figure indicates that all groups showed a progressive increase in food intake as the caloric density of the available diet was reduced. Analysis of variance revealed that for the males there was significant lesion effect, $F(1,4) = 12.21$, $p<0.05$, as well as a significant diet effect, $F(3,12) = 63.35$, $p<0.01$, on food intake. For the females only the effect of diet was statistically significant, $F(3,30) = 28.90$, $p<0.01$. The lesion × diet interaction was not statistically significant for either sex. For both the males and the females a Newman-Keuls post hoc comparison of the intakes on the 4 diets indicated that the level of food intake on each diet was significantly different from the intake on every other diet ($p<0.05$).

The lower panel of this figure shows that the compensatory increase in food intake on the diluted diets was not sufficient to maintain caloric intake at the high fat level for any of the 4 groups. For the males there were significant lesion, $F(1,4) = 14.40$, $p<0.05$, and diet, $F(3,12) = 9.00$, $p<0.05$, effects on caloric intake without a significant interaction. A Newman-Keuls test revealed that caloric intakes on HF and P–O were significantly higher than on the other two diets which did not differ from each other ($p<0.05$). The females showed the same general pattern of response to the diluted diets. There was a significant diet effect, $F(3,30) = 12.54$, $p<0.05$, without significant lesion or interaction effects. A comparison of the over-all means using the Newman-Keuls procedure showed that caloric intake was highest on HF and P–O which did not differ from each other but which were significantly higher than P–25 and P–50 ($p<0.05$). Caloric tracking was not perfect in any of the groups but changes in food intake were always in the appropriate direction and there were no differences between the lesioned and control animals in terms of their responses to the diluted diets.

The upper panel of Fig. 3 shows the changes in water intake on the 4 diets. The control males showed a small increase in water intake as the caloric density of the diet was reduced. The lesioned males showed a large increase in water intake when the diet was switched from HF to P–O and smaller increases as the caloric density of the diet was further reduced. The lesion, $F(1,4) = 16.08$, $p<0.05$, diet, $F(3,12) = 16.07$, $p<0.01$, and the lesion × diet interaction, $F(3,12) = 6.64$, $p<0.05$, were all statistically significant. The interaction was due to the large increase in water intake shown by the lesioned males on the pelleted diets. Water intake for both the lesioned and control females remained relatively constant across diets. One of the lesioned females was grossly polydipsic throughout the experiment, averaging over 140 ml water intake per day. This animal data was omitted from the analyses based on water intake and is not included in the data used to compute the averages for Fig. 3.

The middle panel of this figure shows that the ratio of water intake (ml) to food intake (g) tended to decrease as

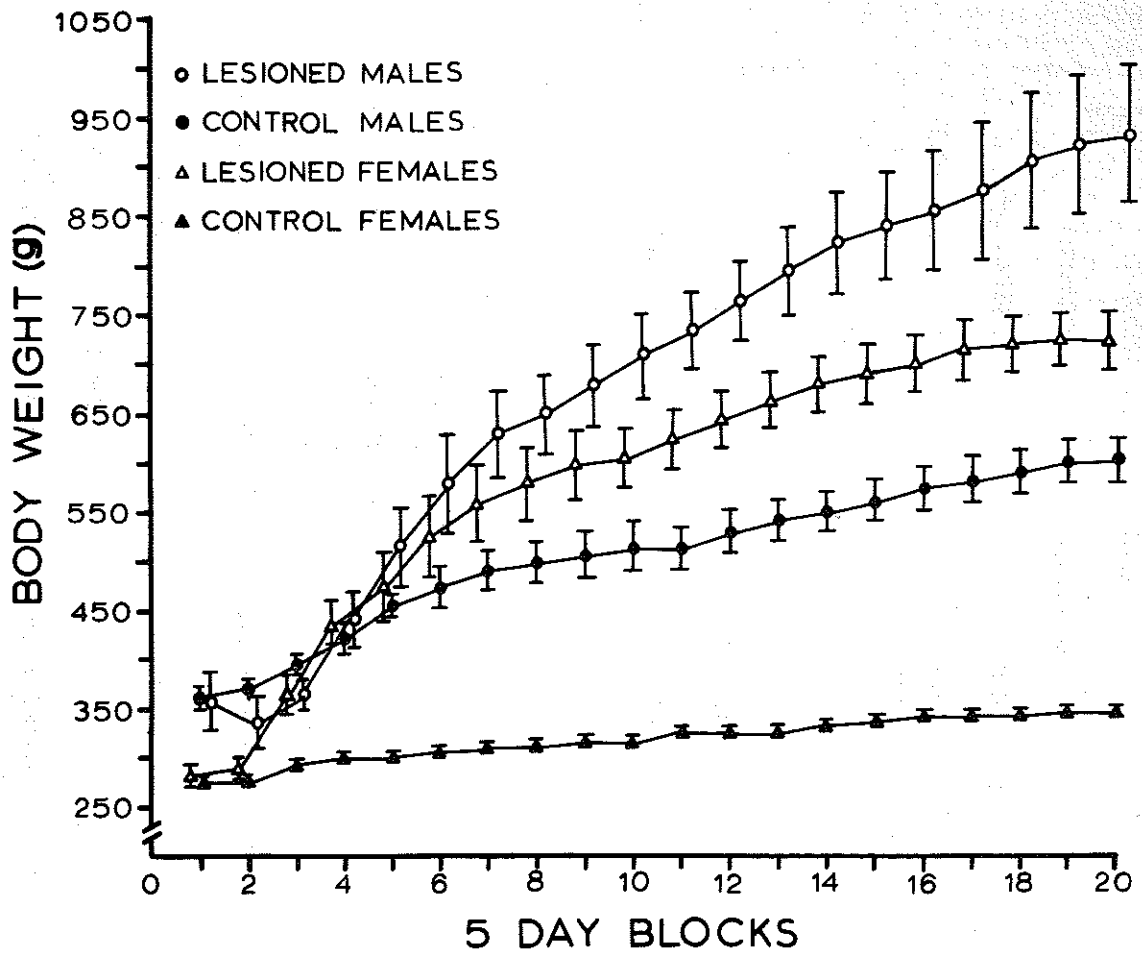

FIG. 1. Mean body weight, after surgery, of male and female lesioned and control animals. Vertical lines with horizontal bars represent standard error of the mean. The points for the lesioned animals are offset slightly to avoid overlapping of points in the graph.

the caloric density of the diet decreased. This decline was statistically significant for both the males, $F(3,12) = 23.58$, $p<0.01$, and the females, $F(3,27) = 11.86$, $p<0.01$. There was no effect of the lesion on this ratio for either the males or the females but for both the males, $F(3,12) = 42.92$, $p<0.01$, and the females, $F(3,27) = 3.90$, $p<0.05$, there was a significant lesion × diet interaction. In both cases the interaction was apparently due to a lower water : food ratio in the lesioned animals on the HF diet.

The bottom panel of this figure reveals that the ratio of water intake (WI) to caloric intake (CI) increased substantially as the caloric density of the diet was reduced. For the males only the diet, $F(3,12) = 48.00$, $p<0.01$, influenced this ratio. For the females both the diet effect, $F(3,12) = 23.09$, $p<0.01$, and the interaction, $F(3,27) = 5.00$, $p<0.01$, were statistically significant. This interaction was due to the large increase in the ratio of water intake to caloric intake shown by the lesioned females on the more dilute diets.

Histology

In general the lesions were large and symmetrical and were very similar to those which produce maximal obesity [8]. Unfortunately 2 of the lesioned males died after completion of the experiment and their brains were unavailable for analysis. The lesions destroyed the ventromedial hypothalamic nuclei (VMN) bilaterally in 3 of the females and the one male. In one female the lesion was unilateral whereas for the other two females the lesion destroyed at least half the VMN bilaterally. The dorsomedial nuclei of the hypothalamus were destroyed in every case. The lesions extended anteriorly to the preoptic nuclei and damaged the paraventricular nuclei in 4 females, and extended into the anterior area and damaged the paraventricular nuclei in one female and the male. In the remaining female damage did not extend beyond the VMN. In all cases the lesions extended ventrally to the surface of the brain and with one exception extended dorsally into the ventral portion of the thalamus. With one exception the lesions extended caudally into the rostral portion of the posterior hypothalamus. Laterally, damage was confined to the VMN in one female, extended to the medial border of the lateral hypothalamus and destroyed the fornix in two females, and extended unilaterally into the lateral hypothalamus in the remaining

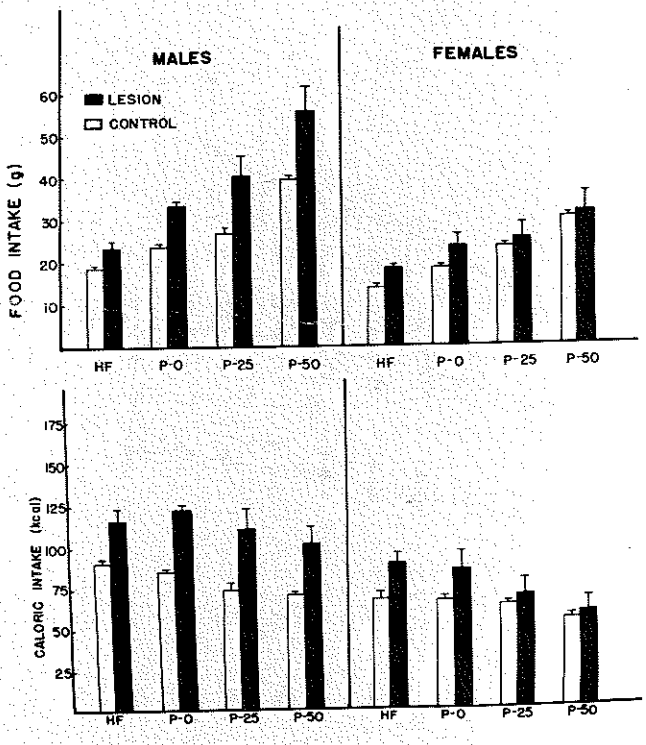

FIG. 2. Mean 24 hr food intake and caloric intake of male and female lesioned and control animals. Vertical lines with horizontal bars represent standard error of the mean.

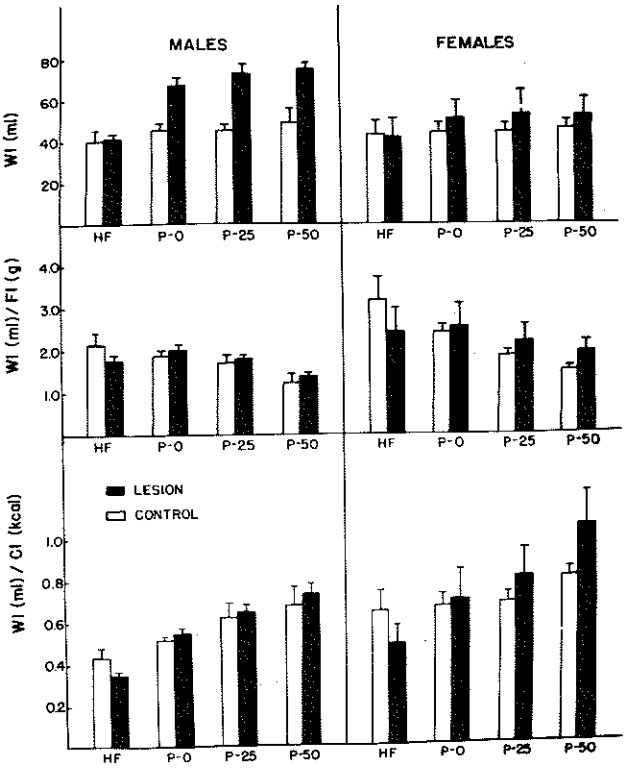

FIG. 3. Mean 24 hr water intake, water intake/food intake, and water intake/caloric intake of male and female lesioned and control animals. Vertical lines with horizontal bars represent standard error of the mean.

three females and the male. There was no apparent relationship between lesion placement and degree of obesity.

DISCUSSION

The major finding of the present experiment was that both male and female rats in the static stage of hypothalamic hyperphagia increase food intake and maintain a relatively constant level of daily energy intake when the caloric density of the available diet is reduced. These results are in contrast to previous findings which have indicated that rats in the static phase of hypothalamic obesity are incapable of adjusting their daily food intake in response to caloric dilution of their diet. For instance, Kennedy [12] found that the food intake of obese rats fell below that of controls when their diet was diluted 50 percent with kaolin. In fact the obese animals ate almost nothing during the first four days on the diluted diet. These results were later confirmed by Teitelbaum [29] and Stominger *et al.* [28] using cellulose as a diluent.

Other studies, however, are in agreement with the present work in showing that animals with lesions of the medial hypothalamus can respond appropriately to the caloric properties of food [4, 28, 30, 32]. Although the diets that were employed in these latter studies differ in almost every conceivable way they share the common feature of being classified as highly palatable. Conversely, failures to demonstrate caloric tracking in hypothalamic hyperphagic animals have all used powdered chow that is made less palatable by the addition of kaolin or cellulose [12, 14, 24, 28, 29]. This pattern of results strongly suggests that the mechanisms for modulating food intake in response to variations in caloric density are intact in the hypothalamic hyperphagic rat but they can be over-ridden by diets or diluents of low palatability.

The preceding analysis emphasizes the potent role of palatability in controlling food intake and adjustment to caloric dilution in hypothalamic hyperphagic rats. However, unless palatability is assessed directly by studying the animal's orosensory responses to the food [13] or indirectly through brief exposure choice behavior [33] it is somewhat circular to attribute changes in food intake to variations in palatability. In two studies the relative preferences of diets on which hypothalamic hyperphagic rats show caloric adjustment have been determined. Carlisle and Stellar [4] found that diets diluted with mineral oil are highly preferred to pellets. Similarly, the three pelleted diets used in the present experiment are all markedly preferred to powdered chow by normal animals even though their caloric density is lower (Hirsch, Jacobs and Dubose, unpublished observations). Although a 24 hour preference test is admittedly not the best technique for evaluating palatability the above evidence does support the assertion that caloric compensation does occur in static hyperphagic rats if preferred diets are used.

Texture appears to be an important dimension of diet palatability. This assertion is supported by previous findings that food intake and final body weights are considerably higher in hypothalamic hyperphagic rats when the same diet is presented as large pellets rather than as a powdered mash [5, 16, 19, 29]. The importance of texture as a

primary factor that contributes to diet palatability is also emphasized by the failure of hypothalamic obese rats to show caloric adjustment to diluted diets when taste is manipulated by adding sucrose or saccharin [14], whereas in the present experiment caloric compensation on the pelleted diets was as accurate in the lesioned animals as it was in the controls.

There is considerable disagreement as to whether hypothalamic hyperphagia is accompanied by deficits in water metabolism. A relative hypodipsia that is reflected in lower water : food ratios [4, 5, 15, 20, 25] and is accompanied by elevated levels of serum sodium [26] is frequently reported to coexist with hypothalamic hyperphagia. There are also many reports that fail to note any changes in the water : food ratio [3, 6, 18, 19, 24, 27]. The results of the present study suggest that the type of diet that is employed may be one factor that contributes to these discrepant results. In the present experiment the lesioned animals had a lower water : food ratio on only the high fat diet. On the pelleted diets this value was comparable to the control ratio or was slightly elevated. This deficit is almost always reported on high fat diets but occurs with a much lower frequency in studies that use chow or pellets with their lower fat levels [4, 5, 6, 15, 19, 20, 25, 27].

Although it is generally agreed that in the long-term the normal animal drinks sufficient water to maintain minimal urine and fecal volumes for solute excretion [17] the relative importance of food bulk and calories on daily water intake is not clear. Adolph [1] showed that water intake is more closely related to the amount of nutrients consumed than to the amount of solids on kaolin diluted diets. In the present experiment the water : food ratio tended to decrease and the water : calories ratio tended to increase as the diet became more dilute. The direction and magnitude of these changes were quite similar for the hyperphagic and control animals. No clear interpretation of these trends can be given in this study, however, because changes in the caloric densities of the diets are confounded with variations in fat, protein, carbohydrate and mineral levels which influence water balance in different ways [17,23].

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